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Baby care product development: Artificial urine *in vitro* assay is useful for cosmetic product assessment

Arnaud Degouy, Marie-Pierre Gomez-Berrada, Pierre-Jacques Ferret *

Safety Assessment Department, Pierre Fabre Dermo Cosmetique, Vigoulet Auzil, France

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ABSTRACT

As a result of infants' inability to control urination, the skin of the diaper area has special needs for protection from irritating effects of urine and prevention of diaper dermatitis such as products for cleansing and protection of the skin. Several *in vitro* models are currently available to assess tolerance. *In vitro* testing using artificial urine allows the protective effects of diaper-region cosmetics to be ascertained. Thus, a new model defined as "artificial urine *in vitro* assay" has been added to our traditional pre-clinical *in vitro* testing program. IL1-α is a highly active and pleiotropic pro-inflammatory cytokine. It plays a key role in inflammation and is the biological mirror of irritation induced by diaper dermatitis. This study determines, on human skin explants, if a cosmetic formula is (1) tolerated equally as well in the presence of artificial urine as in its absence and (2) is able to decrease IL1-α production induced by artificial urine or Sodium Dodecyl Sulfate. 31 tests including 17 in-house formulas, 10 bench-markers and 4 combinations of products were performed and data obtained are represented on a simple four-point scale (from practically non protective to very protective). It allows determination of formula-type groups that will have predictable protective properties in subsequent clinical trials and comparison with competitors' products. It is a useful aid in the formulation stage and provides readily-useable data for the cosmetic risk assessment.

1. Introduction

As a result of infants' inability to control urination, the skin of the diaper area has special needs for protection from irritating effects of urine and prevention of diaper dermatitis. Diaper rash or Irritant Diaper Dermatitis (IDD) is a non specific medical term that describes a spectrum of symptoms in the diaper area caused by inflammatory skin reactions that occurs regularly specifically between the ages of 6–12 months (Heimall et al., 2012). It is the consequence of an interaction of several factors among which one of the most important is a prolonged contact of the skin with a mixture of urine and faeces. As stated, urine can increase the permeability of diapered skin to irritants and can directly irritate skin when exposure is prolonged (Berg et al., 1986). There is an increasing recognition that gentle cleansing, good diaper practice and regular application of a protective barrier are all essential elements in the prevention of IDD (Lund et al., 1999; Putet et al., 2001; Adam, 2008). When considering what would constitute the best barrier preparation, one needs to consider the tolerability, safety and efficacy. Several *in vitro* models are currently available to assess skin or mucous tolerance, and *in vitro* testing using artificial urine allows the protective effects of diaper-region cosmetics to be ascertained. Thus, a new model developed by Ephyscience and defined as "artificial urine *in vitro* assay" was added to our traditional pre-clinical *in vitro* testing program. It allows us to determine, on human skin explants, if a cosmetic formula is (1) tolerated equally as well in the presence of artificial urine as in its absence and (2) is able to decrease IL1-α production induced by artificial urine or SDS.

2. Materials and methods

2.1. Materials

Tests were performed on 17 in-house formulae and 10 bench-markers formulae alone or in association. The aim and type of the tested products and formulae were identified and described in Table 1. All chemicals were purchased from Sigma–Aldrich (Saint Louis, MO, USA.), excepted Albumin purchased from Fisher

Abbreviations: C, combination; BM, bench marker formula; CAP, continuous aqueous phase; COP, continuous oily phase; IDD, irritant diaper dermatitis; IH, in-house formula; IL1-α, interleukin 1-α; PPNP, para-nitrophenylphosphate.

* Corresponding author. Address: Pierre Fabre Dermo Cosmetique, Allée Camille Soula – BP74, Castanet Tolosan-Cedex, Vigoulet Auzil 31322, France. Tel.: +33 5 61 75 52 69/01 49 10 82 86; fax: +33 5 61 75 52 52.

E-mail address: pierre-jacques.ferret@pierre-fabre.com (P.-J. Ferret).

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Table 1
Set of the 27 products used alone or in combination in the present study.

Products	Aim and type of the product		Type and nature of formula
In-house formula			
IH_1	Cl	LO	Cleansing milk (CAP)
IH_2	Cl	RO	Cleansing milk (CAP)
IH_3	T	LO	Spray (COP)
IH_4	T	LO	Liniment (COP)
IH_5	T	LO	Emulsion (CAP)
IH_6	T	LO	Paste (CAP)
IH_7	Cl	LO	Cleansing milk (CAP)
IH_8	Cl	LO	Cleansing milk (CAP)
IH_9	Cl	LO	Cleansing water (CAP)
IH_{10}	T	LO	Ointment (COP)
IH_{11}	T	LO	Cream (CAP)
IH_{12}	T	LO	Cream (COP)
IH_{13}	Cl	LO	Cleansing oil (COP)
IH_{14}	T	LO	Paste (COP)
IH_{15}	T	LO	Cream (COP)
IH_{16}	T	LO	Cream (COP)
IH_{17}	T	LO	Lotion (CAP)
Bench-markers formula			
BM_1	T	LO	Liniment (COP)
BM_2	T	LO	Paste
BM_3	T	LO	Paste
BM_4	T	LO	Paste (COP)
BM_5	T	LO	Cream (COP)
BM_6	T	LO	Emulsion (COP)
BM_7	T	LO	Emulsion (COP)
BM_8	T	LO	Ointment
BM_9	T	LO	Paste
BM_{10}	T	LO	Ointment
Combinations			
C_1	Cl + T	LO	Cleansing oil + paste
C_2	Cl + T	LO	Cleansing water + paste
C_3	Cl + T	LO	Cleansing milk + paste
C_4	Cl + T	LO	Cleansing milk + cream

IH, in-house formula; BM, bench-marker formula; C, combination ($C_1 = IH_{13} + IH_{14}$, $C_2 = IH_9 + IH_{14}$, $C_3 = IH_7 + IH_{14}$, $C_4 = IH_8 + IH_{11}$); Cl, cleansing product; T, treating product; LO, leave-on product; RO, rinse-off product; CAP, continuous aqueous phase; COP, continuous oily phase.

Scientific (Illkirch, France). Dulbecco's Modified Eagle Medium (DMEM) and HAM's F12 were purchased from PAA (PAA Laboratories, France), whereas foetal calf serum (FCS), penicillin/streptomycin and Amphotericin B were purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Methods

Laboratory Ephyscience (Carquefou, France) has developed the "Artificial urine *in vitro* assay" for assessing on human explants the tolerance and the efficacy of the tested product to inhibit IL-1 production after exposure with artificial urine. Artificial urine was prepared according to the formula of Shmaefsky (1990, 1995) as follow: urea (18.2 g/L), sodium chloride (7.5 g/L), potassium chloride (4.5 g/L), sodium phosphate (4.8 g/L), creatinin (2 g/L), albumin (50 mg/L), and pH is adjusted at 5.1.

Human skin from healthy donors was received in adherence to the Declaration of Helsinki principles as residual material from plastic surgery with local ethics committee approval. 12 abdominoplasties from donors (11 females and 1 male) aged an average of 39 years (the group ranged in age from 28 to 55 years old) were used to provide explants. Full-thickness membranes were prepared by removing any extraneous fat and subcutaneous tissue from the underside of the skin by blunt dissection and treated with PBS and ethanol 70%. 3 Skin punch biopsies (8 mm diameter) were taken per experiment and maintained in DMEM/HAM's F12 (50:50), supplemented with FCS (10%) and penicillin/streptomycin and Amphotericin B (1% and 0.4% respectively) for 24 h at 37 °C, under 5% CO_2. After 24 h of incubation, explants were exposed or not to urine like ±urease (1 UI/ml), SDS 0.5% ±dexamethasone (10 μM), at 20 μl per explant, in presence or in absence of the tested product, as described Fig. 1. SDS was used as a positive control for the induction of IL-1α, whereas dexamethasone previously diluted at 10 μM in the medium, was used as an inhibitor of IL-1α production. 24 h after incubation, media were removed and frozen at −20 °C until IL-1α dosage, and viability was assessed according to Yang et al., 1996 by intracellular phosphatase spectrophotometric dosage. Para-nitrophenylphosphate is transformed in para-nitrophenol by viable cells intracellular phosphatases. Para-nitrophenol absorbance at 405 nm is directly proportional to the number of viable cells.

IL-1α was quantified in the incubation media using a commercially available assay kit (R&D systems, Abingdon, United

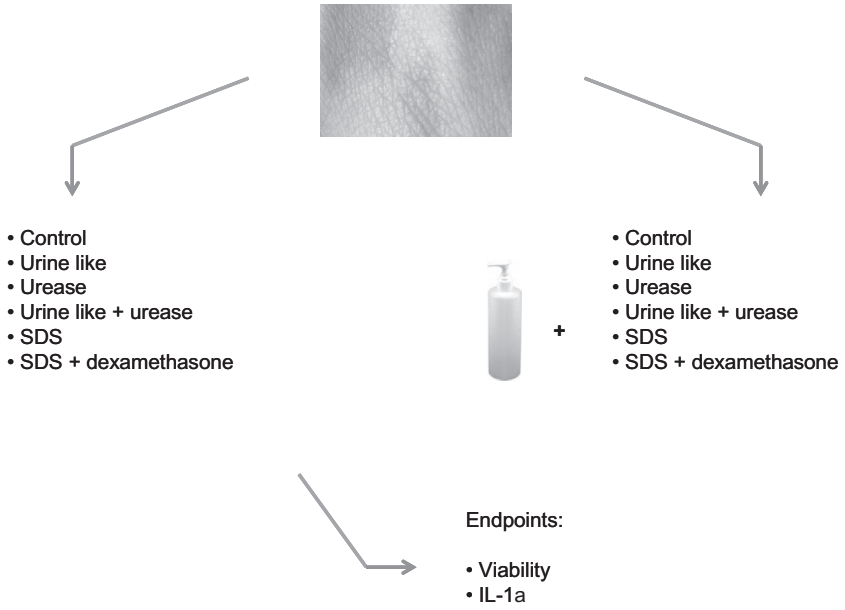

Fig. 1. Schematic representation of the artificial urine *in vitro* assay. Human skin punch biopsies (8 mm diameter) were exposed or not to urine like ±urease (1 UI/ml), SDS 0.5% ±dexamethasone (10 μM), at 20 μl per explant, in presence or in absence of the tested product. SDS was used as a positive control for IL-1α induction, whereas dexamethasone was used as an inhibitor of IL-1α production. 24 h after incubation, media were removed and frozen at −20 °C until IL-1α dosage, and viability was assessed.